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Quantitation of Protein Turnover in the Human Adult Lens Using the 14C Bomb-Pulse

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Disclaimer

This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

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FINAL PROGRESS REPORT

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Grant Number: 5R21EY18722-2
Project Title: Quantitation of protein turnover in the human adult lens using the 14C bomb-pulse
Grantee Organization: Lawrence Livermore National Laboratory
Project Period: 1 September 2008 – 31 August 2012
PD/PI: Bruce Buchholz

Summary
The project used the temporal variation in environmental $^{14}C/C$ since 1955 to probe the turnover of carbon in proteins of the human lens. We investigated whether different protein fractions possess different ages using ^{14}C bomb pulse. Adult human lenses obtained from tissue banks were concentrically dissected by gently removing cell layers in water or shaved to the nucleus with a curved micrometer controlled blade. Cells were lysed and proteins were separated into water-soluble and water-insoluble fractions. Small molecules were removed by the use of 3 kDa spin filters. The $^{14}C/C$ was measured in paired protein fractions by accelerator mass spectrometry and an average age was assigned using the ^{14}C bomb pulse. Layers and lens nuclei were also analyzed by protein MS to determine the major proteins in the samples. Proteins were separated by HPLC and elutions were pooled in an attempt to produce samples large enough for isotope analysis.

The distribution of water-soluble proteins changed with age as we moved from out layers (young cells) to inner layers (old cells). The most striking change in soluble protein measurement with aging was the near complete loss of βB2 in the nucleus. Other crystalline proteins that experienced relative losses with age were βA3, βA4, and αA. The variation of in the distribution of protein expression with cell age is the topic of a paper in preparation by N. Nguyen based on his dissertation data.

The mass of water-soluble proteins was distributed across about 20 different proteins. From previous experience, we knew that gel separation would not for isotope analyses of separated proteins. The gels can never be completely removed from the protein and the petroleum-derived carbon in the gels skews isotope ratios. Proteins needed to be kept in the liquid phase for separations. A micro-rotofor could not adequately separate the proteins and high levels of carbon in proprietary buffers resisted complete removal. A HPLC was used that could separate proteins, but its recovery efficiency was too low to acquire samples large enough for isotope analysis. The quantity of recoverable purified individual proteins was too small to make $^{14}C/C$ analyses as intended in specific aim #1 of the proposal.

Specific aim #2 of the proposal was to elucidate the trends for the rate of protein turnover among the chronologically stratified regions of healthy human lenses and investigate bomb carbon incorporation into the lens nucleus of donors born after the 1963 peak of the ^{14}C bomb-

pulse or who reached middle age prior to 1955. We were unable to obtain viable lenses of donors who had reached middle age before 1955 (born before 1915). We did obtain many lenses from donors 60-85 years old. The water-insoluble fractions possessed $^{14}C/C$ ratios consistent with the age of the cells. The water-soluble fractions contained carbon younger than the paired water-insoluble fraction in all cases. The amount of new carbon in proteins was consistent with annual carbon turnover of about 1% throughout life. A single younger, donor had a larger difference between soluble and insoluble carbon isotope ratios, consistent with 2-3% annual turnover, suggesting that carbon turnover may decrease or cease with age. As the first direct evidence for carbon turnover in protein from adult human nuclear fiber cells, this discovery supports the emerging view of the lens nucleus as a dynamic system capable of maintaining homeostasis in part by protein production or repair and intricate transport mechanisms. This finding implies that the lens plays an active role in the aversion of age related nuclear (ARN) cataract through protein production or repair.

Publications

Falso MJS, Buchholz BA. Bomb Pulse Biology. Nuclear Instruments & Methods in Physics Research. Section B. (2012) in press. NIHMS408176.
http://dx.doi.org/10.1016/j.nimb.2012.08.045

Stewart DN, Lango J, Nambiar KP, Falso MJS, Fitzgerald PG, Rocke DM, Hammock BD, Buchholz BA. Carbon Turnover in Water-Soluble Protein of the Adult Human Lens. Molecular Vision (2013) Submitted 24 September 2012, Comments received 5 November 2012. Revision in preparation for resubmission December 2012. NIHMS and PMCID not yet issued.

Nguyen NN, Nambiar KP, Lango J, Fitzgerald PG, Buchholz BA, Changes in Protein Expression with Age Between Layers of Individual Adult Human Lenses. (2013) in preparation.

Dissertation

Nguyen NN. Distribution and Characterization of Human Eye Lens Proteins. Ph.D. Dissertation. University of California, Davis, CA. December 2011.